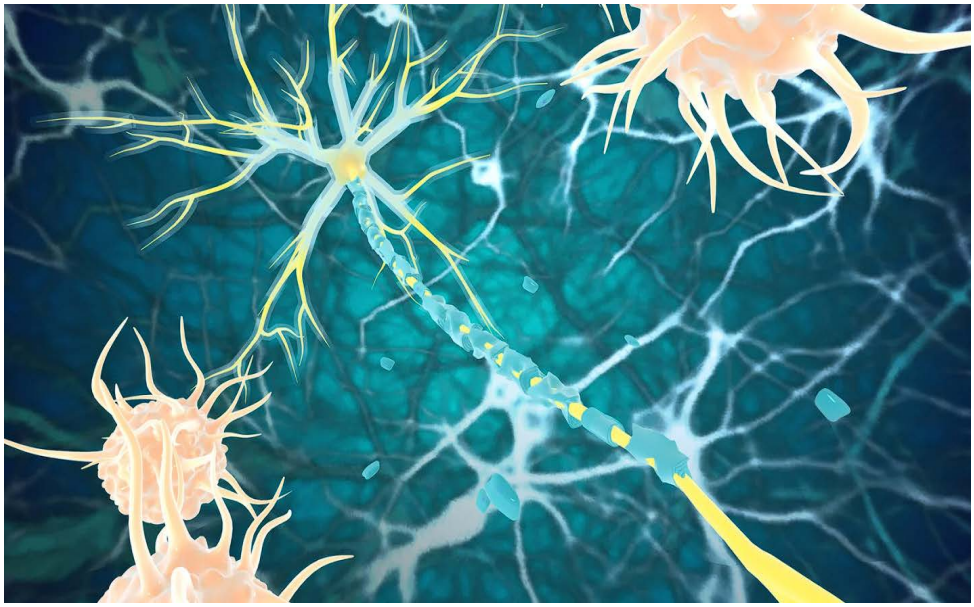
Berkeley Pharma Tech
Journal of Medicine

Anti-Inflammatory Interventions for Autism Spectrum Disorder

By: Haily Holston, Avani Karvat, Simran Lallian, PingJu Wu



Correspondence:
holstoncalifornia@gmail.com

Keywords:
Autism Spectrum Disorder, Neuroinflammation, Mesenchymal STEM Cell Therapy, Oxytocin, Resveratrol, Sulforaphane, Combination Therapy

Abstract

One of the defining factors of Autism Spectrum Disorder (ASD) is neuroinflammation, which may be targeted to find effective therapies in managing ASD symptoms. Some promising treatments include mesenchymal STEM cell (MSCs) therapy, cxytocin (OT), sulforaphane (SFN), and resveratrol (RSV). MSCs are located in many parts of the body that can reduce secondary neurodegeneration and neuroinflammation while promoting neurogenesis and angiogenesis. OT is a hormone that moderates social and emotional communication, bonding, and social learning, while also having profound anti-inflammatory effects. SFN is a naturally occurring compound in cruciferous vegetables, such as broccoli and sprouts, and activates a transcription factor which regulates anti-inflammatory and antioxidant genes. RSV is found in plants, such as grapes and berries, and helps stabilize the central and peripheral immune response and oxidative stress markers, subsequently reducing neuroinflammation. All of these treatments have shown promising potential, but it is abundantly clear that further research is needed in addition to combined therapies. Since ASD is a spectrum, not every case can be treated the exact same way. By targeting neuroinflammation, we can address the root cause of ASD rather than the symptoms.

Submitted August 11, 2023
Accepted April 5, 2024

Published June 28, 2024

Full Open Access

1. Introduction

ASD affects 1 in 36 children as of 2020,[1] but the cause of the disorder is still unknown. While there is no clear consensus in the scientific community on the specific mechanism that leads to the development of ASD, some theories include genetic predispositions or environmental factors.[2] However, neuroinflammation has been consistently found in individuals diagnosed with ASD. Some of the key characteristics of ASD are poorly developed social skills, difficulty with expressive and receptive communication, and the presence of restrictive and repetitive behaviors.[3] There is no current cure for ASD which poses a significant call for action and attention to novel treatment.

There are many pharmacological therapies prescribed for ASD such as antipsychotics, hormones, CNS stimulants, and antidepressants to name a few.[4] However, these interventions only target the symptoms of ASD instead of neuroinflammation, and out of the symptoms only one aspect of ASD such as aggression, repetitive behaviors, hyperactivity/inattention, or social behavior. It is very difficult to find treatments that cater to all the symptoms, for they mainly focus on accommodating symptoms and limitations associated with ASD such as speech, language, and learning deficits. 75% of individuals with ASD also suffer from other associated disorders such as attention-deficit hyperactivity disorder (ADHD), anxiety, bipolar disorder, depression, and many others.[5] Non-pharmacological therapies that mediate symptoms of ASD include behavioral management treatment, cognitive behavior therapy, social skills training, and speech and language therapy. However, the focus of this review will be on novel forms of anti-inflammatory treatments such as mesenchymal STEM cell therapy, oxytocin, sulforaphane, and resveratrol, which have been successful in reducing neuroinflammation in animals and humans, providing synaptic protection, and relieving symptoms of ASD.

2. Pathophysiology of ASD and Neuroinflammation

A single cause of ASD is yet to be found, but there are a large range of possible factors that play a significant role in its onset, like genetics, environmental influences.

Neuroinflammation is found to be an underlying factor of ASD and other neurodevelopmental disorders. Although this correlation is apparent, it is still unclear whether neuroinflammation is a symptom of ASD *or* if ASD is a result of neuroinflammation.[6] Neuroinflammation refers to an inflammatory response taking place in the central nervous system (CNS). At low levels, neuroinflammatory signaling is critical for learning and memory functions, but at higher or chronic levels from CNS injuries, it can be a causing factor of neurodegenerative diseases and even aging.[6]

The neuroinflammatory response involves microglia cells in the CNS and their polar nature. In ASD patients, neuroinflammation has been observed in the cerebellum in early developmental stages and continues to the later stages of their lives.[6] Neuroinflammation can be detected through an increase in microglial cell density and somal volume in the white matter of the brain.[6] Once microglia are activated, there are two inflammatory pathway options:the pro-inflammatory response in the M1 phenotype and the anti-inflammatory response in the M2 phenotype.[6] Pro-inflammatory cytokines that commonly increase in patients with ASD are *IL-6*, *TNF-a*, *GMCSF*, and *IL-8,* while those that decrease are *TGF-β* and *IL-10.*[7] Furthermore, chronic glial activation of the pro-inflammatory pathway leads to the overall inflammatory response being altered.[8]

Additionally, transforming growth factors (TFGβ 1, 2, 3) play vital roles as regulators in the immune system and general homeostasis including the regulation of inflammation. For instance, ASD patients with decreasing behavioral measures have been found to have decreased TFGβ plasma.[7]

An environmental factor, maternal immune activation (MIA), is one of the most significant factors linked to ASD in children. MIA triggers inflammation of the placenta and neuroinflammation in the mother.[9] MIA can increase IL-17A expression, which leads to neuron cell death and thus hinders normal social behaviors as found in embryonic mouse brains.[9] Other ASD symptoms are found to be induced by MIA including anxiety-related repetitive behaviors.[9] Neuroinflammation caused by MIA has also been correlated to oxidative stress, another factor in causing ASD. Oxidative stress triggers negative feedback leading to unnatural and

disrupted brain development, which is a key factor ASD and other neurodevelopmental disorders.[9]

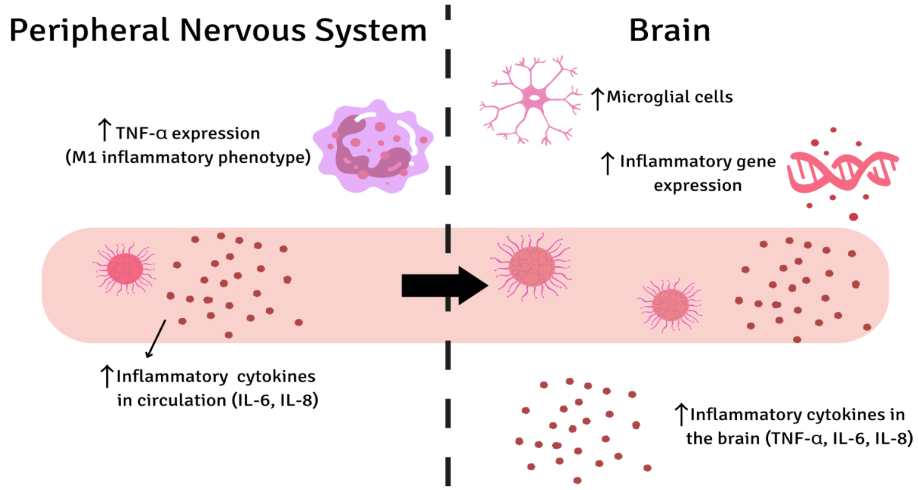


Figure 1: The inflammatory response in the central nervous system and brain is mediated by genes and cytokines.[10] The increased TNF-alpha gene expression leads to the release of pro-inflammatory cytokines in the PNS.

3. Anti-Inflammatory Interventions

3.1 Mesenchymal STEM Cell Therapy

MSCs are stem cells that can be found in any postnatal tissue, including the brain, it can perform a variety of functions, such as reducing secondary neurodegeneration and neuroinflammation, or promoting neurogenesis and angiogenesis.[11] The popularity of MSC therapy is due to its ability to be harvested from various locations in the body, being easy to culture in lab, having little ethical considerations, and being well-tolerated when implanted into patients.[6] In addition, there are no significant safety concerns during infusion or after.[12]

The way MSCs function is through bidirectional immunomodulatory effects, caused by direct contact that increase inflammation when the immune system is under-active and decrease inflammation when it is over-active.[6] Secondly, MSCs secrete extracellular vesicles (EVs), growth factors, chemokines, and cytokines.[6] EVs are known to be essential for

regulating the inflammatory response, mediating cell communication, and transmitting mediators during inflammation to ensure the anti-inflammatory response.[13] Growth factors are hormone-like proteins such as nerve growth factors (NGFs) in the brain, that can help suppress inflammation and switch the immune response to anti-inflammatory.[14] Cytokines and chemokines are proteins that help regulate the immune response; when they are not in control it can lead to neuroinflammation, neurodegeneration, and demyelination of the CNS and PNS.[15]

Pre-clinical studies demonstrate the potential of MSC therapy. An in vitro study demonstrated that MSCs modulate neuroinflammation through significant reduction of mRNA expression of proinflammatory cytokines in microglia.[16] MSCs were harvested from male mouse tibia and fibula, then tested with BV2 and primary microglia isolated from mouse pup brains.[16] Another study utilized in vitro culture exosomes from MSCs derived from human umbilical cords (hUC), which proceeded to be administered intranasally into mice. There was improved sociability and decreased repetitive behaviors in mice treated with valproic acid, which closely mimics ASD.[12]

A clinical study was conducted on 37 children with ASD from ages 3-14 years old, during which umbilical cord-derived mesenchymal stem cells (UCMSC) in conjunction with human cord blood mononuclear cells (CBMNCs) were tested. The subjects were divided into three groups in a non-randomized, open-label, single center phase I/II trial, which are most suitable for establishing medication dosages with the highest efficacy.[17] The CBMNC group consisted of 14 participants, who all received a transfusion of CBMNCs and rehabilitation therapy. The 9 participants in the combination group received both CBMNC and UCMSC transfusions, as well as rehabilitation therapy (9 subjects). The control group, which also had fourteen participants, received only rehabilitation therapy. The CBMNC group had significant results in comparison to the control group, however the combination group had the most significant results based on three scales: the Aberrant Behaviour Checklist (ABC), Clinical Global Impression scale (CGI) and Childhood Autism Rating Scale (CARS), with no significant safety issues.[17] This study demonstrated the effectiveness of both MSC therapy and the impact of dual therapy.

Another clinical trial observed the impact of intravenous (IV) infusions of human cord tissue mesenchymal stromal cells (hCT-MSCs) in 12 children with ASD from ages 4 to 9 through an open-label, phase I study.[18] The hCT-MSCs were provided by a third party manufacturer, and each child underwent one, two, or three doses with 2 month intervals in between. Clinical and laboratory assessments were conducted in person initially and at the 6-month mark for a baseline, then remotely again at the 12-month point following the last infusion. Upon the end of the study, 50% of all the children across all groups showed signs of improvement in at least 2 ASD measures. The tests used to gauge efficacy were the Vineland Adaptive Behavior Scale, Pervasive Developmental Disorder-Behavior Inventory, and Clinical Global Impression Scale. It is uncertain whether this was only due to the treatment, but it was concluded that hCT-MSCs improves communication and socialization in ASD patients. Once the trial treatment was also determined to be well-tolerated and safe for children, hCT-MSCs were successfully manufactured.[18] However, it is important to note further trials are necessary to confirm the long-term effects and safety of the treatment for the general population.[18]

In summary, the benefits of MSC therapy is limited to improvement of symptoms without reversing the condition itself, making it a promising supplementary treatment for managing ASD.[12] More research on a larger scale is necessary to understand the full extent of the treatment's efficacy in managing varying degrees of ASD symptoms and characteristics.

3.2 Oxytocin

Oxytocin (OT), or the "social hormone", is a neuropeptide naturally produced by the human body. It is most widely known for its ability to moderate social and emotional communication, bonding, and learning in the human brain. Oxytocin is produced in the brain's hypothalamus and is released into the bloodstream by different regions of the brain, such as the pituitary gland, and the spinal cord.

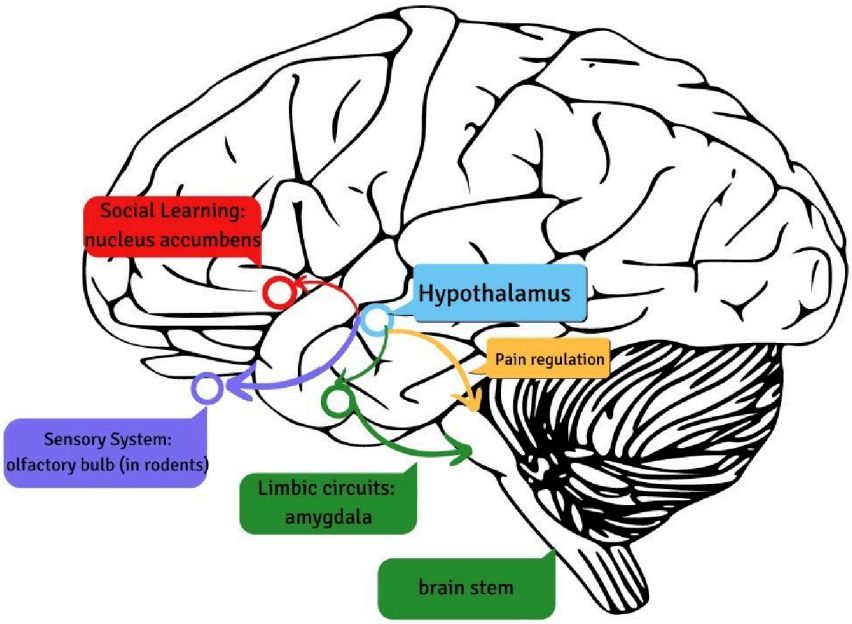


Figure 2: Map of the key pathways by which oxytocin modulates social functioning in the brain. Oxytocin influences the modulation of sensory input (olfactory system in rodents), social learning through interactions with serotonin systems in the nucleus accumbens, and amygdala and limbic circuit activity. It also directly affects the brainstem nuclei to promote bonding, trust, and social interactions.[19]

Oxytocin has profound anti-inflammatory effects in multiple organ systems, including but not limited to the inhibition of the immune system's activation of inflammatory cells, significant reduction of NADPH oxidase and ROS production, and the lowering of pro-inflammatory cytokine production and neurotoxicity in the nervous system.[20] A 2008 study researching the role of oxytocin's anti-inflammatory properties conducted trials with OT and lipopolysaccharide (LPS) treatments through both individual and combination therapies. The results found that in 10 healthy men who received the treatments, through individual or combination therapies, there was long-term reduction of endotoxin-induced macrophage inflammatory protein-1-alpha, macrophage inflammatory protein-1-beta, cortisol, and VEGF levels, among other proteins responsible for generating inflammatory responses in the body.[21] Most notably, OT had a significant effect on decreasing neuroendocrine cell and cytokine expression; these are two components that play a vital role in cell signaling between the nervous system and other organs that coordinate the body's inflammatory immune response. Oxytocin's moderation of the release of these proteins has been

shown to relieve inflammation in the body. In conclusion, oxytocin demonstrates noteworthy therapeutic potential for ASD not only because of its anti-inflammatory properties, but because of the vital role the hormone plays in the development of complex social behaviors.[22]

In mouse models of ASD, oxytocin exhibits an increase in social recognition and restoration of social behavior. Rodents, like most animals, rely on efficient extraction of sensory information using their olfactory system to shape their behaviors and perceive the world around them. A study was conducted to test whether OT would trigger the main olfactory system in female adult Wistar rats to process social odor cues. The experiment found that after invoking the release of endogenous OT in mice with an olfaction-OT social recognition task, the mice that released endogenous OT had longer anogenital investigation events in comparison to the control rats. In rodents, anogenital investigation is vital for olfactory sampling and social recognition; the rats with prompted endogenous OT release took more time to investigate and draw social conclusions about their surroundings.[23] Another study observing the restoration of social behavior in a Cntnap2 mouse model of ASD after exogeneous OT release concluded that the treated mice with previously low sociability demonstrated stronger preference to interact with other mice after OT treatment.[24] Therefore, studies tests on mouse models demonstrated how both endogenous and exogenous OT release prompts heightened social engagement and learning.

Clinical trials testing the role of OT in relieving social deficits in patients with ASD have been vital to our understanding of OT's capacity as a potential therapy. One of such studies was a randomized, double blinded, and placebo controlled clinical trial observing probiotic and oxytocin combination therapy in patients with ASD aged 3-20 years old. The patients were separated into two groups: 1 placebo group and 1 probiotic group. The groups were observed for 28 weeks and at week 16, OT was introduced to both. The results indicated trends of improvement in scores based on the study's Aberrant Behavior Checklist, Social Responsiveness Scale, and stereotypic behavior score in the probiotic and OT combination group, as well as significant CGI improvement.[25]

Another clinical trial explored the effect of OT nasal spray on social interaction deficits observed in young children with autism. The study administered the OT nasal spray in the morning and night over a 5-week trial period, and concluded that it was both well-tolerated by children and improved caregiver-rated social responsiveness in comparison to the children in the placebo group.[26] This study noted that children with ASD found this method preferable and significantly more tolerable, providing valuable insight into the future testing of exogenous OT in younger patients.

Though the success of oxytocin in both relieving inflammation throughout the body and promoting social processing and engagement is apparent, one of the limitations of oxytocin as a therapy for ASD is the inconsistency of studies on oxytocin treatment in humans. Not all clinical trials show significant behavior changes or relief from repetitive behaviors and social deficits in individuals with ASD.[27]

3.3 Sulforaphane

Sulforaphane (SFN) is a naturally occurring compound abundant in cruciferous vegetables, such as broccoli and sprouts, and recent research has demonstrated its efficacy in alleviating symptoms associated with ASD.[28] SFN's therapeutic effects stem from its ability to activate a master transcription factor known as nuclear factor erythroid 2 related factor (Nrf2). Nrf2 plays a pivotal role in regulating anti-inflammatory and antioxidant genes, making it crucial for the body's defense against oxidative stress and inflammation.[29] While the exact underlying mechanism remains uncertain, evidence from a rodent-model study suggests that sulforaphane's activation of Nrf2 is likely to address elevated Th17 immune responses and oxidative stress observed in individuals with ASD.[30] As a result, this correction of immune dysfunction and oxidative stress is expected to lead to a reduction in ASD symptoms.

Several clinical trials have been conducted to explore the effects of SFN treatment in ASD, yielding encouraging results. In one placebo-controlled, double-blind, randomized trial done by Singh et al., 29 young men with moderate to severe ASD received daily oral doses of SFN for 18 weeks.[31] The results demonstrated substantial and reversible improvements in behavior,

as quantified by widely accepted measures completed by parents/caregivers and physicians. SFN's mechanism of action lies in its ability to upregulate genes that protect cells against oxidative stress, inflammation, and DNA damage, all of which are prominent characteristics associated with ASD. Even though two participants encountered unprovoked seizures following treatment, it's essential to emphasize that SFN exhibited minimal toxicity, making it a comparatively safe option for therapeutic use.

Another clinical trial investigated the effects of adjuvant treatment with SFN and risperidone in alleviating irritability in 60 children with ASD.[32] The combination of SFN and risperidone led to greater improvements in irritability and hyperactivity/noncompliance symptoms compared to the placebo group. These results support the safety and efficacy of SFN as an adjuvant treatment for behavioral improvements in children with ASD.

Additionally, a larger randomized clinical trial with 108 subjects in China further substantiated the potential of SFN treatment.[33] Clinician-rated scales showed a significant improvement in the SFN group, with one-third of participants experiencing a significant decrease in scores after 12 weeks of treatment. SFN was well-tolerated across all age groups, including young children, and its effects appeared to be greater in participants over 10 years of age. However, inconsistencies between caregiver and clinician-rated scales indicate the need for more clinical trials to confirm and refine the findings.

While SFN treatment holds promising potential for addressing ASD symptoms, it also has limitations. Not all clinical trials have yielded statistically significant improvements in behavior, and the effects may vary depending on age groups and assessment methods. In some studies, SFN treatment did not show significant clinical improvement in the behavioral outcome measures evaluated in children with ASD.[34, 35] Moreover, the sample sizes in some trials were limited, which may impact the generalizability of the findings. Further research with larger cohorts is necessary to better understand SFN's effects and determine the optimal dosages and treatment duration for different age groups and severity levels of ASD.

The need to validate the responses to SFN treatment in ASD has led to studies exploring potential biomarkers. These candidate molecular markers,

associated with ASD in three physiological pathways, include cytoprotective enzymes, heat shock proteins, and pro-inflammatory markers.[36] Ex vivo experiments using peripheral blood mononuclear cells (PBMCs) from healthy subjects showed that all markers exhibited quantifiability, accuracy, and reproducibility after SFN treatment. When administered orally to ASD patients, SFN led to an increase in cytoprotective enzymes and heat shock proteins, while pro-inflammatory markers decreased. These encouraging results indicate that these markers have the potential to be utilized as guidance for the development of SFN interventions for ASD.

In a nutshell, the promising potential of SFN treatment in ASD offers hope for the development of mechanism-based therapeutic approaches. SFN's ability to modulate oxidative stress and inflammation highlights its relevance in addressing the underlying pathophysiology of ASD. Nevertheless, additional clinical trials along with biomarker establishment are required to address the limitations and validate the consistency and robustness of SFN's effects on ASD symptoms.

3.4 Resveratrol

Resveratrol (RSV) is a polyphenolic stilbenoid acting as a phytoalexin that has been found to reduce common symptoms of ASD in animal models due to its anti-inflammatory and anti-oxidative properties.[37] RSV is naturally made in plants, such as grapes and berries, as they respond to pathogen attacks.[37]

RSV can decrease neuroinflammation by inhibiting activation of the pro-inflammtory pathway and the proinflammatory cytokine release.[38, 39] *TNF-a* and MMP-9 levels significantly decrease in the presence of RSV, therefore reducing neuroinflammation[39]. MMP-9 stimulates proinflammatory cytokines as well as processes the NLGN3 (neuroligin) and NRXN1 (neurexin) genes that are linked to ASD[39]. MicroRNA-155 (MiR-15) also increases microglia's inflammatory response, which is decreased by RSV to reduce neuroinflammation.[29]

RSV has shown its effectiveness in reducing neuroinflammation in several studies differing in models used, dosage amounts, and methods of administration. RSV admitted orally (5, 10, 15 mg/kg) was found to

decrease pro-inflammatory cytokine concentrations such as *IL-6* and *TNF-a*, which in turn counteracts neuroinflammatory markers.[37] In a study performed with RSV being injected at 0 hours, 8 hours, and 18 hours, RSV was found to increase the anti-inflammatory M2 phenotype polarization and reduce the M1 pro-inflammatory response.[29]

The valproic acid model (VPA), a fatty acid used as an antiepileptic drug and mood stabilizer, is often used to study ASD.[37] VPA is also a potent teratogen, leading to abnormalities in embryonic development which can induce ASD.[38] VPA exposure during pregnancy has been associated with ASD in offspring and can cause developmental neurotoxicity in the child's central nervous system.[37]

In a study with RSV administration of 3.6 mg/kg for 12-13 days, there was a significant prevention and reduction of social deficits of ASD in the VPA model[37]. In this model, RSV decreased negative effects of ASD to the nest-seeking behavior of the rats; however, RSV didn't have an effect on latency to decision making[37].

In a separate study with the VPA model with rats, RSV was found to prevent impairments in reciprocal social interaction[40]. Nose-to-nose sniffing habits of rats were also significantly different when treated with RSV[40]. Decreased decision accuracy caused in the VPA model was also prevented with RSV[41]. However, RSV couldn't prevent food preference changes or the behavior of repetitive self-grooming as it was affected by VPA exposure[40].

In addition to RSV's success in the VPA model, in the BTBR model RSV reduced persistent self-grooming, a repetitive habit in rats with ASD, with doses of 20-40 mg/kg[37]. In human patients with ASD, these repetitive behaviors include fidgeting or sniffing. In the BTBR mice, CCR and CXCR, chemokine receptors related to inflammation, were significantly higher, but decreased with RSV treatment[37].

In the propanoic acid (PPA) model, studies found the association of MMP activation to inflammatory cytokines and mitochondrial dysfunction[39]. With this PPA model, RSV has proven to improve modifications of rats

with ASD because of its properties, specifically being anti-*TNF-a* and anti-MMP-9[39].

Further studies and clinical trials can help to further investigate RSV's effectiveness against neuroinflammation and in turn, symptoms of ASD. Additional research will also be able to find how to utilize RSV while minimizing side effects such as fetal abnormalities[37].

4. Practical Applications

4.1 Mesenchymal STEM Cell Therapy

There is a large potential for MSC Therapy because it is very easy to obtain through bone marrow, adipose tissue, placenta, skin, umbilical cord blood, umbilical cord perivascular cells, umbilical cord Wharton's jelly, amniotic fluid, breast milk, and more[42]. MSCs are easy to isolate and expand, and are unique due to their self-renewal and differentiation properties[42]. These cells are also able to cross the blood-brain-barrier which helps it migrate to sites of tissue injury and inflammation.

Although there are numerous proof of concept studies, there is a significant shortage of clinical trials and other therapies researching practical use of MSCs. Further investigation of the MSCs as a potential therapy for ASD is necessary to determine the effectiveness on patients with varying severities. Since most clinical trials have studied young children, study participants within a wider range of age groups would significantly increase our understanding of MSCs and adult patients with ASD.

Furthermore, the efficacy of this therapy based on MSCs different tissue types and varying administration methods has yet to be explored[42]. Researchers are unsure whether systematic delivery (ex: intravenous) is enough to reach the brain, compared to direct implantation, or intranasal administration which is non-invasive[42]. Additionally, it is unclear whether MSCs obtained from different sources in the body will have differing or similar therapeutic effectiveness[42].

ASD is also associated with a myriad of other autoimmune conditions such as autoimmune thyroiditis, rheumatoid arthritis, ulcerative colitis, celiac disease, and type 1 diabetes[42]. There is potential to investigate the use of MSCs in helping manage these conditions alongside symptoms of ASD, however many of these are either in the pre-clinical or early clinical trial phases.

Although ASD still remains without a cure, MSC therapy emerges as an intervention with great potential as MSCs can benefit synaptic health and have the potential to target tissue damage, regeneration/repair, inflammation, and ultimately aid in addressing the underlying pathology of neuroinflammation[42]. MSCs can be transplanted directly with no genetic modification, or pretreatment, can differentiate itself, and don't have any significant side effects such as tumors[42].

4.2 Oxytocin

OT has tremendous therapeutic potential for ASD specifically because of its role in promoting social learning and bonding, as well as its anti-inflammatory properties in various systems in the body[22]. The neuropeptide has been highly studied throughout the past few decades to investigate how it may relieve one of ASD's most notable characteristics: social deficit. Studies have shown that in both animals and humans, OT has the capacity to heighten social problem solving skills and increase social engagement in both patients with ASD and rodent models of autism.

Although there are a plethora of successful clinical trials exploring OT and the social changes it creates in ASD patients, further research of OT in humans is necessary in order to develop useful therapies. While OT shows great promise for increasing sociability in patients with ASD, many clinical trials in humans show inconsistent results in treatments using OT alone[27]. Most notably, there are many unsuccessful clinical trials investigating OT where there have been no significant increases in social engagement or a decrease in repetitive behaviors in patients with ASD[27].

The strength of OT lies in combination therapy. When used with probiotics simultaneously, OT treatment has shown drastic increases in the patients' caregiver rated sociability and decreases in repetitive behavior

patterns[25]. Further research, especially exploring successful combinations of OT and other potential therapies, is necessary to better understand how to target and relieve specific symptoms of ASD.

Lastly, OT demonstrates therapeutic capability for ASD due to its method of administration. OT is produced in the brain's hypothalamus, it is one of the body's naturally occurring neuropeptides. Exogenous OT treatments are commonly distributed intranasally, and this method has been reported to be tolerable by children and show low levels of uncomfortability in patients.

The human body's OT can also be evoked to collaborate in treatment methods[26].

Some limitations of the trials using OT are that only healthy men were included as participants, and no women were involved.[21] Additionally, the proposed half-life of OT was only around 20 minutes in the mammalian brain[25], the small sample size limited the ability to effectively analyze subgroups, population heterogeneity led to subjects with varying treatment responses, and cultural and language barriers potentially influenced behavioral assessments[26].

Overall, OT shows great potential in helping patients with ASD boost social awareness, increase social engagement and social behaviors and decrease repetitive behaviors patterns. The neuropeptide's capability to collaborate with other treatment methods such as probiotics in combination therapy must be further researched to determine the most useful amalgamation to relieve the most notable characteristics revolving around the social deficits of ASD.

4.3 Sulforaphane

Further research is essential to determine the most suitable dosage of SFN tailored to individuals with varying body weights. The emerging evidence regarding SFN's favorable effects on alleviating symptoms associated with ASD is undeniably promising, underscoring the need for additional exploration to unveil the most effective dosing regimen that maximizes its therapeutic benefits while minimizing potential adverse effects.

Studies conducted thus far have reported encouraging outcomes in individuals with ASD who have consistently integrated SFN into their dietary routine[31, 32, 33]. This naturally occurring compound, abundantly found in various food sources such as broccoli, brussels sprouts, and cabbage, provides a convenient and readily accessible method of supplementation[28]. Given its ubiquitous presence in everyday foods, SFN represents a safe option for oral consumption, enhancing its appeal as a potential treatment for ASD.

SFN possesses multifaceted attributes that position it as an exceptionally promising candidate in the search for effective therapies for ASD. Beyond its easy availability, SFN boasts various pharmacological properties that contribute to its therapeutic potential. As a potent antioxidant, SFN combats oxidative stress, a common feature associated with ASD[43, 44]. Additionally, it exhibits anti-inflammatory effects that can alleviate neuroinflammation, frequently observed in individuals with autism[8, 45, 46].

Furthermore, SFN's impact on the body's detoxification mechanisms is noteworthy. By enhancing phase II detoxification enzymes, it facilitates the elimination of harmful substances and toxins, further promoting the overall health and well-being of individuals with ASD[47, 48].

SFN's versatility reaches beyond its capacity as a standalone therapy. Considering that individuals with ASD can derive benefits from personalized combinations of treatments and services, there is a significant opportunity to synergize SFN with other compounds or medications[33, 34]. Such integration has the potential to enhance SFN's therapeutic impact on ASD symptoms, presenting a promising pathway for future therapeutic interventions and optimizing its overall efficacy.

While these developments are encouraging, it is crucial to acknowledge that research in the field of autism is continuously evolving. Delving deeper into the complexities of ASD and exploring the precise mechanisms through which SFN exerts its effects will enable us to refine treatment protocols better, catering to individual needs more effectively.

Overall, SFN's potential as a therapeutic agent for ASD shines brightly. Its accessibility, presumed safety, and beneficial effects on alleviating symptoms

have garnered significant attention from the scientific community. As further investigations are conducted to establish ideal dosages and explore potential synergistic effects with other treatments, the path towards harnessing the full potential of SFN in enhancing the lives of individuals with ASD becomes closer. This dedicated pursuit represents a crucial step towards offering comprehensive and effective solutions to those living with this neurodevelopmental condition.

4.4 Resveratrol

Because RSV can cross the brain-blood barrier, studying its effects is helpful in investigating neurodevelopmental disorders[37]. Due to this characteristic, RSV has low concentrations in the brain and high concentrations in the blood, meaning RSV isn't very effective when administered orally with only a 1% bioavailability due to how easily it is absorbed and excreted[49]. This low bioavailability makes RSV more beneficial while being used in combination with other therapies[49].

Polyphenols, including RSV, aren't naturally synthesized in animals, and thus plant-rich diets are a more effective way to achieve its effects[49]. Foods with high amounts of RSV include grapes, peanuts, and plums in addition to several other recommended options[31].

Even with RSV's high potential to be used as an additional therapeutic agent to reduce and improve the symptoms of ASD on a biochemical, molecular, and behavioral scale, more research must be done to effectively utilize its abilities[39]. More trials and research can help to better understand RSV's mechanisms of action in various conditions[50].

It is important to note the participants of most RSV studies are predominantly male animals, leaving room for questions regarding how females may experience different interactions with

RSV for ASD symptoms[37]. There have been studies with evidence of differences in levels of estrogen receptors in ASD patients and with limited knowledge of how sex hormones play a role in RSV, this is a future area if study necessary to further prove RSV's effectiveness[37].

A notable negative effect of RSV's use is particularly on pregnant women because of fetal birth defects as shown in a study with Japanese macaques[37]. The risk to pregnant women may also be accompanied with symptoms of diarrhea and nausea[31]. Further study of these effects is beneficial to provide better care with less risk.

RSV shows promising results to patients with ASD particularly because of its ability to reduce neuroinflammation. With the goal of alleviating the symptoms of ASD these patients face, further research of RSV in combination with other therapeutic options would be extremely beneficial. Even with this further research, it is crucial to acknowledge the diversity of ASD patients and their symptoms, leading to evolving information about this treatment.

5. Future Directions

MSC, OT, SFN, and RSV are all anti-inflammatory treatments that have demonstrated their individual capacities to relieve inflammation in the body, namely neuroinflammation, one of the hallmark underlying factors of ASD. Each of these treatments have their own strengths and weaknesses, for example OT shows significant promise in increasing social engagement and social problem solving in patients but has inconsistent results in humans when not used in combination therapy. One discrepancy to note is that OT has shown consistent measurable increases in sociability when administered intranasally in mice, yet when administered intranasally in children, there is great variation in results. In future, more research with other methods of distribution of OT may lead to more consistent results and higher levels of sociability in human patients with ASD. Further research to investigate the capacity of each of these therapies in combination must be explored in order to gauge their combined therapeutic potential. Combination therapies that utilize the strong points of MSC, OT, SFN, and RSV must be studied in order to eventually create individualized therapies for ASD patients that are specifically designed for that patient's symptoms and deficits. Since ASD is a spectrum, individualized combination treatment therapy holds immense promise in alleviating each patients' most notable

symptoms. Currently, there is no literature specifically exploring MSCs, OT, RSV, and SFN in combination to alleviate symptoms of ASD. Exploring these therapies in combination with current treatments, especially probiotic treatments, is necessary to create effective and tolerable treatments to reduce neuro-inflammation in patients with ASD.

6. Conclusions

Research of potential therapies for Autism Spectrum Disorder has significantly advanced in recent years as scientists explore new remedies for the symptoms and underlying causes of ASD. However, current treatments focus on alleviating common symptoms of ASD rather than targeting the foundational causes of ASD such as neuroinflammation. Although there is no cure for ASD, treatments targeting both neuroinflammation and critical symptoms of ASD such as

Mesenchymal STEM Cell Therapy (MSC), Oxytocin (OT), Sulforaphane (SFN), and Resveratrol (RSV) show immense therapeutic potential. Most notably, ASD is a spectrum. No individual therapy studied thus far has the capacity to be effective in all individuals with ASD. Potential interventions serve to resolve some of the overarching consequences of ASD, particularly neuroinflammation, and social deficits such as repetitive behavior patterns, poorly developed social skills, and difficulty with expressive and receptive communication. It is imperative that further research studying the efficiency of individualized combination therapy of MSC, OT, SFN, and RSV be explored to create the most effective medicinal combinations for individuals with ASD.

References

1. Maenner MJ, et al. Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2020. *MMWR Surveill Summ.* 2023;72(2):1-14. doi:10.15585/mmwr.ss7202a1.

2. Hodges H, Fealko C, Soares N. Autism spectrum disorder: definition, epidemiology, causes, and clinical evaluation. *Transl Pediatr.* 2020;9(Suppl 1). doi:10.21037/tp.2019.09.09.

3. Faras H, Al Ateeqi N, Tidmarsh L. Autism spectrum disorders. *Ann Saudi Med.* 2010;30(4):295-300. doi:10.4103/0256-4947.65261.

4. LeClerc S, Easley D. Pharmacological therapies for autism spectrum disorder: a review. *P T.* 2015;40(6):389-397.

5. Sharma SR, Gonda X, Tarazi FI. Autism Spectrum Disorder: Classification, diagnosis and therapy. *Pharmacol Ther.* 2018;190:91-104. doi:10.1016/j.pharmthera.2018.05.007.

6. Wong RSY. Neuroinflammation in autism spectrum disorders: potential target for mesenchymal stem cell-based therapy. *Egypt J Neurol Psychiatry Neurosurg.* 2022;58:91. doi:10.1186/s41983-022-00525-2.

7. Ohja, Kshama et al. Neuroimmunologic and Neurotrophic Interactions in Autism Spectrum Disorders: Relationship to Neuroinflammation. *Neuromolecular Med.* 2018;20(2):161-173. doi:10.1007/s12017-018-8488-8.

8. Eissa, N., Sadeq, A., Sasse, A., Sadek, B. Role of Neuroinflammation in Autism Spectrum Disorder and the Emergence of Brain Histaminergic System. Lessons Also for BPSD?. *Front Pharmacol.* 2020;11:886. doi:10.3389/fphar.2020.00886.

9. Usui, Noriyoshi et al. Neuroinflammation and Oxidative Stress in the Pathogenesis of Autism Spectrum Disorder. *Int J Mol Sci.* 2023;24(6):5487. doi:10.3390/ijms24065487.

10. Hughes, HK et al. Innate immune dysfunction and neuroinflammation in autism spectrum disorder (ASD). *Brain Behav Immun.* 2023;108:245-254. doi:10.1016/j.bbi.2022.12.001.

11. Das, M., Mayilsamy, K., Mohapatra, SS, Mohapatra, S. Mesenchymal stem cell therapy for the treatment of traumatic brain injury: progress and prospects. *Rev Neurosci.* 2019;30(8):839-855. doi:10.1515/revneuro-2019-0002.

12. Liang, Duan et al. Mesenchymal Stem Cell-Derived Exosomes for Treatment of Autism Spectrum Disorder. *ACS Appl Bio Mater.* 2020;3(9):6384-6393. doi:10.1021/acsabm.0c00831.

13. Spiers JG, Vassileff N, Hill AF. Neuroinflammatory Modulation of Extracellular Vesicle Biogenesis and Cargo Loading. *Neuromolecular Med.* 2022;24(4):385-391. doi:10.1007/s12017-022-08704-3

14. Villoslada P, Genain CP. Role of nerve growth factor and other trophic factors in brain inflammation. *Prog Brain Res.* 2004;146:403-414.

doi:10.1016/S0079-6123(03)46025-1

15. Ramesh G, MacLean AG, Philipp MT. Cytokines and chemokines at the crossroads of neuroinflammation, neurodegeneration, and neuropathic pain. *Mediators Inflamm.* 2013;2013:480739. doi:10.1155/2013/480739

16. Ooi YY, Dheen ST, Sam Wah Tay S. Paracrine effects of mesenchymal stem cells-conditioned medium on microglial cytokines expression and nitric oxide production. *Neuroimmunomodulation.* 2015;22(4):233-242. doi:10.1159/000365483

17. Lv YT, Zhang Y, Liu M, et al. Transplantation of human cord blood mononuclear cells and umbilical cord-derived mesenchymal stem cells in autism. *J Transl Med.* 2013;11:196. doi:10.1186/1479-5876-11-196

18. Sun JM, et al. Infusion of human umbilical cord tissue mesenchymal stromal cells in children with autism spectrum disorder. *Stem Cells Transl Med.* 2020;9(10):1137-1146. doi:10.1002/sctm.19-0434

19. Fineberg SK, Ross DA. Oxytocin and the Social Brain. *Biological Psychiatry*. 2017;81(3). doi:https://doi.org/10.1016/j.biopsych.2016.11.004

20. Mehdi SF, Pusapati S, Khenhrani RR, et al. Oxytocin and Related Peptide Hormones: Candidate Anti-Inflammatory Therapy in Early Stages of Sepsis. *Frontiers in Immunology*. 2022;13. doi:https://doi.org/10.3389/fimmu.2022.864007

21. Clodi M, Vila G, Geyeregger R, et al. Oxytocin alleviates the neuroendocrine and cytokine response to bacterial endotoxin in healthy men. *American Journal of Physiology-Endocrinology and Metabolism*. 2008;295(3). doi:https://doi.org/10.1152/ajpendo.90263.2008

22. Gonzalez A, Hammock EAD. Oxytocin and microglia in the development of social behaviour. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences*. 2022;377(1858):20210059. doi:https://doi.org/10.1098/rstb.2021.0059

23. Oettl, L.-L., Ravi, N., Schneider, M., et al. Oxytocin enhances social recognition by modulating cortical control of early olfactory processing. *Neuron*. 2016, May 4. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4860033/

24. Peñagarikano O, Lázaro MT, Lu XH, et al. Exogenous and evoked oxytocin restores social behavior in the CNTNAP2 mouse model of autism. *Science Translational Medicine*. https://pubmed.ncbi.nlm.nih.gov/25609168/

25. Kong XJ, Liu J, Liu K, et al. Probiotic and oxytocin combination therapy in patients with autism spectrum disorder: A randomized, double-blinded, placebo-controlled pilot trial. *Nutrients*. 2021;13(5):1552. https://doi.org/10.3390/nu13051552

26. Yatawara CJ, Einfeld SL, Hickie IB, Davenport TA, Guastella AJ. The effect of oxytocin nasal spray on social interaction deficits observed in young children with autism: a randomized clinical crossover trial. *Molecular Psychiatry*. 2015;21(9):1225-1231. https://doi.org/10.1038/mp.2015.162

27. Sikich L, Kolevzon A, King BH, et al.

Intranasal oxytocin in children and adolescents with autism spectrum disorder. *New England Journal of Medicine*. 2021;385(16):1462-1473. https://doi.org/10.1056/nejmoa2103583

28. Rajavel T, Devi KP. Phytochemicals as epigenetic modifiers for cancer management with special reference to lung cancer. *Epigenetics of Cancer Prevention*. Published online 2019:271-286. doi:10.1016/b978-0-12-812494-9.00013-5

29. Ma Q. Role of NRF2 in oxidative stress and toxicity. *Annual Review of Pharmacology and Toxicology*. 2013;53(1):401-426. doi:10.1146/annurev-pharmtox-011112-140320

30. Nadeem A, Ahmad SF, Al-Harbi NO, et al. Nrf2 activator, sulforaphane ameliorates autism-like symptoms through suppression of th17 related signaling and rectification of oxidant-antioxidant imbalance in periphery and brain of BTBR t+TF/J mice. *Behavioural Brain Research*. 2019;364:213-224. doi:10.1016/j.bbr.2019.02.031

31. Singh K, Connors SL, Macklin EA, et al. Sulforaphane treatment of autism spectrum disorder (ASD). *Proceedings of the National Academy of Sciences*. 2014;111(43):15550-15555. doi:10.1073/pnas.1416940111

32. Momtazmanesh S, Amirimoghaddam-Yazdi Z, Moghaddam HS, Mohammadi MR, Akhondzadeh S. Sulforaphane as an adjunctive treatment for irritability in children with autism spectrum disorder: A randomized, double-blind, placebo-controlled clinical trial. *Psychiatry and Clinical Neurosciences*. 2020;74(7):398-405. doi:10.1111/pcn.13016

33. Ou J, Smith RC, Tobe RH, et al. Efficacy of sulforaphane in treatment of children with autism spectrum disorder: A randomized double-blind placebo-controlled multi-center trial. *Journal of Autism and Developmental Disorders*. Published online 2022. doi:10.1007/s10803-022-05784-9

34. Magner M, Thorová K, Župová V, et al. Sulforaphane treatment in children with autism: A prospective randomized double-blind study. *Nutrients*. 2023;15(3):718. doi:10.3390/nu15030718

35. Zimmerman AW, Singh K, Connors SL, et al. Randomized controlled trial of sulforaphane and metabolite discovery in children with autism spectrum disorder. *Molecular Autism*. 2021;12(1). doi:10.1186/s13229-021-00447-5

36. Liu H, Zimmerman AW, Singh K, et al. Biomarker exploration in human peripheral blood mononuclear cells for monitoring sulforaphane treatment responses in autism spectrum disorder. *Scientific Reports*. 2020;10(1). doi:10.1038/s41598-020-62714-4

37. Malaguarnera M, et al. Resveratrol in autism spectrum disorders: Behavioral and molecular effects. *Antioxidants (Basel, Switzerland)*. 2020;9(3):188. doi:10.3390/antiox9030188

38. Taleb A, et al. Emerging mechanisms of valproic acid-induced neurotoxic events in autism and its implications for pharmacological treatment. *Biomedicine & Pharmacotherapy*. 2021;137:111322. doi:10.1016/j.biopha.2021.111322

39. Bhandari R, Kuhad A. Resveratrol suppresses neuroinflammation in the experimental paradigm of autism spectrum

disorders. *Neurochemistry International*. 2017;8-23.

40. Hirsch MM, Zimian LB, Florentino A, et al. Behavioral alterations in autism model induced by valproic acid and translational analysis of circulating microRNA. *Food Chem Toxicol*. 2018;115:336-343. doi:10.1016/j.fct.2018.02.06

41. Fontes-Dutra M, de Oliveira A, da Rosa Borges M, et al. Resveratrol Prevents Cellular and Behavioral Sensory Alterations in the Animal Model of Autism Induced by Valproic Acid. *Front Synaptic Neurosci*. 2018;10:9. doi:10.3389/fnsyn.2018.00009

42. Liu Q, Chen MX, Sun L, et al. Rational use of mesenchymal stem cells in the treatment of autism spectrum disorders. *World J Stem Cells*. 2019;11(2):55-72. doi:10.4252/wjsc.v11.i2.55

43. McGuinness G, Kim Y. Sulforaphane treatment for autism spectrum disorder: A systematic review. *EXCLI J*. 2020;19:892-903. doi:10.17179/excli2020-2487

44. Liu X, Lin J, Zhang H, et al. Oxidative Stress in Autism Spectrum Disorder-Current Progress of Mechanisms and Biomarkers. *Front Psychiatry*. 2022;13:813304. doi:10.3389/fpsyt.2022.813304

45. Houghton CA. Sulforaphane: Its "Coming of Age" as a Clinically Relevant Nutraceutical in the Prevention and Treatment of Chronic Disease. *Oxid Med Cell Longev*. 2019;2019:2716870. doi:10.1155/2019/2716870

46. Townsend BE, Johnson RW. Sulforaphane induces Nrf2 target genes and attenuates inflammatory gene expression in microglia from brain of young adult and aged mice. *Exp Gerontol*. 2016;73:42-48. doi:10.1016/j.exger.2015.11.004

47. Riedl MA, Saxon A, Diaz-Sanchez D. Oral sulforaphane increases Phase II antioxidant enzymes in the human upper airway. *Clin Immunol*. 2009;130(3):244-251. doi:10.1016/j.clim.2008.10.007

48. Wen H, Yang HJ, An YJ, et al. Enhanced phase II detoxification contributes to beneficial effects of dietary restriction as revealed by multi-platform metabolomics studies. *Molecular & Cellular Proteomics: MCP*. 2013;12(3):575-586. https://doi.org/10.1074/mcp.M112.021352

49. Sawda C, Moussa C, Turner RS. Resveratrol for Alzheimer's disease. *Annals of the New York Academy of Sciences*. 2017;1403:142-149. https://doi.org/10.1111/nyas.13437

50. Malaguarnera L. Influence of Resveratrol on the Immune Response. *Nutrients*. 2019;11(5):946. doi:10.3390/nu11050946